morphology deficiencies are hallmark of neurodevelopemental and neurodegenerative disorders such as Autism, Fragile X - syndrome and Down's syndrome. A better understanding of spine morphogenesis can provide clues to the development of novel clinical therapies. Dendritic filopodia are the immediate precursors to spines during development. Therefore as a first step in our study, we analyzed filopodial motility in hippocampal neurons. The effect of a variety of experimental cytoskeletal drug treatments could be predicted with a simple 1D mechano-chemical model of filopodial dynamics. We then focused on three central aspects of spine morphogenesis: metamorphosis of filopodia into spines, diversity of spine shapes and their stability. To gain insight into the regulation of spine shape, we developed a 2D mechano-chemical model of spinogenesis where the actin network was modeled as a viscoelastic fluid within moving boundaries. The 2D model was then used to identify parameters and filopodia motility profiles that steer spinogenesis towards stable shapes. (supported by NIH grant P41 GM103313).

2149-Plat

Traction Force Microscopy Based on an Active Cable Network Model

Jerome Soine[1], Christoph Brand[1], Jonathan Stricker[2], Patrick W. Oakes[2], Margaret L. Gardel[2], **Ulrich S. Schwarz**[1].
[1]Institute for Theoretical Physics, Heidelberg University, Heidelberg, Germany, [2]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL, USA.

Generation of mechanical force is essential for the function of tissue cells, for example during migration, wound healing or rigidity sensing. The traction field of adherent cells can be measured on soft elastic substrates using traction force microscopy (TFM). However, mathematical reconstruction of traction fields effectively requires inversion of the long-ranged elastic equations and therefore is an ill-posed inverse problem. Moreover measurement of the traction pattern alone does not tell us how force is distributed inside the cell. To improve the robustness, resolution and scope of conventional TFM, we have developed a new procedure called model-based TFM (MBTFM). We estimate the distribution of intracellular tension from elastic substrate data by minimizing the difference between the experimentally measured displacement field and the predictions of a detailed theoretical model based on active cable networks. Previously this type of model has been successfully used to predict cell shapes on micropatterned substrates. For MBTFM, we consider not only active network contraction, but also contributions of various types of contractile bundles modeled as contractile one-dimensional line element embedded into the network. Subsequent computer simulations of network contraction and parameter optimization allow us to estimate the most likely distribution of tension over various contractile structures and adhesion sites.

2150-Plat

Migration, Force Generation and Mechanosensing of Cells in Collagen Gels

Julian Steinwachs[1], Claus Metzner[1], Katerina Aifantis[2], Ben Fabry[1].
[1]University Erlangen Nuremberg, Erlangen, Germany, [2]University of Arizona, Tucson, AZ, USA.

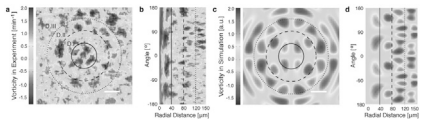
Collagen gels are frequently used to study cell migration in a three-dimensional environment. Mechanical properties of collagen gels are governed by non-affine deformation of the collagen fibrils, such as buckling and tautening, resulting at the macroscopic scale in strain stiffening under shear and a strong lateral contraction under stretch. It is currently unknown how these macroscopic properties play out at the scale of a migrating cell, and how this depends on the cell geometry. To explore this question, we develop a non-linear elastic material model for collagen gels based on observations from confocal microscopy that fibrils can evade mechanical stress using their internal degrees of freedom. This non-affine behavior results in a non-linear force length relationship of fibril segments and leads to a macroscopic strain stiffening and lateral contraction. In particular, we show that tautening of fibrils results in a strong material stiffening against expanding forces, e.g. from a migrating cell with a diameter larger than the network pore diameter. By this mechanism, even a soft collagen gel can sterically constrain a migrating cell. Using our material model, we compute cell traction forces, induced stresses as well as material stiffening, from collagen fiber displacements during the migration of MDA-MB 231 breast carcinoma cells through dilute (0.6 mg/ml, Young's modulus 85 Pa) and dense (2.4 mg/ml, Young's modulus 1100 Pa) collagen gels. We find that cells exert highly localized forces onto the matrix, leading to a localized ~2-fold material stiffening. However, the average traction force magnitude increases with collagen concentration by ~3-fold, from 40nN in dilute gels to 120nN in dense gels. This observation may help explain why cells can migrate more efficiently in stiffer gels, despite their narrower pore diameter.

2151-Plat

Cell Divisions Cause Long-Range Well-Ordered Vorticity Patterns in Endothelial Tissue

Ninna S. Rossen, Jens M. Tarp, Joachim Mathiesen, Mogens H. Jensen, **Lene Oddershede**.
Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark.

Endothelial cells line the blood vessels of the circulatory system and adhere tightly to their neighbors to prevent leakage, thus causing the cells to move within the endothelial monolayer i a highly collectively fashion. In healthy blood vessels with a laminar blood flow, the endothelial cell division rate is low, only sufficient to replace apoptotic cells. The division rate significantly increases during embryonic development and under halted or turbulent flow. Using PIV analysis we investigated the long-range dynamics induced by cell division in an endothelial monolayer under non-flow conditions, thus mimicking the conditions during vessel formation or around blood clots. A cell division locally injects energy into the system and strongly influences monolayer dynamics. We show that cell divisions induce long-range, well-ordered vortex patterns extending several cell diameters away from the division site. This is rather surprising considering the system's low Reynolds number. Our experimental results are reproduced by a hydrodynamic continuum model simulating division as a local pressure increase in a non-nematic, meso-scale turbulent state. The long-range physical communication may be crucial for embryonic development and for healing endothelial tissue around blood clots.



Platform: Intrinsically Disordered Proteins

2152-Plat

Prediction of the Effects of the Val66Met Polymorphism on the Conformational Ensemble of an Intrinsically Disordered Protein, Brain-Derived Neurotrophic Factor

Ruchi Lohia, Reza Salari, Grace Brannigan.
Computational Biology, Rutgers University, Camden, NJ, USA.

The discovery of Intrinsically Disordered Proteins (IDP) has challenged the structure-function paradigm and forced us to find new ways for identifying functional mechanisms of proteins. Studies show that IDPs can function while being partly disordered or may fold once they bind to their receptors. Disease-associated Single Nucleotide Polymorphisms (SNP) are common in the disordered regions of proteins, but not much is known about their effect on the protein structure. Brain Derived Neurotrophic Factor (BDNF) belongs to the family of neurotrophins, and facilitates neurogenesis in its short (mature) form but apoptosis in its long (pro) form. A common (found in 4% of the United States population) SNP that results in the Val66Met mutation in the disordered N terminus domain of the long form of BDNF (proBDNF) has been associated with various neuropsychiatric disorders such as bipolar disorder and Parkinson's and Alzheimer's diseases. In order to explore the effect of this SNP on protein structure and dynamics, we conducted Molecular dynamics simulations to identify the effect of the above SNP on likely conformations of proBDNF. Although IDPs have been identified to change their conformations rapidly, many also exhibit some residual secondary structure, which might be biased towards the bound conformation. To construct the ensemble of proBDNF in both forms, large-scale fully atomistic replica exchange calculations of both the Val and Met forms of proBDNF were carried out. We find significant differences in the secondary structure available to Val and Met forms of the protein in the region surrounding the SNP, with results that agree with recent NMR studies. This suggests a position specific residue-type dependence of the residual secondary structure of proBDNF, which might account for functional compromise.

2153-Plat

How Do Interactions in Cis with Ordered Domains Influence Sequence-Ensemble Relationships of Intrinsically Disordered Regions?

Anuradha Mittal, Kanchan Garai, Rohit V. Pappu.
Washington University in St Louis, St Louis, MO, USA.

The conformational properties of intrinsically disordered proteins (IDPs) are governed by their amino acid sequences. An overwhelming majority of disordered regions are often found in cis with ordered domains as terminal tails or inter-domain linkers. The motivating question is if the rules governing sequence-ensemble relationships that have been inferred for IDPs as autonomous units apply for describing the conformational properties of disordered

regions in cis to ordered domains. Using atomistic simulations and fluorescence studies we characterize the conformational properties of several archetypes of intrinsically disordered sequences in the presence and absence of cis-acting ordered domains. In particular, we quantify the effects of sequence composition, chain length, and sequence patterning of disordered sequences on the inter-domain coupling. The balance between intra-domain and inter-domain interactions can modulate intrinsic conformational propensities of the disordered regions. The circumstances giving rise to convergence toward generic random-coil behavior for disordered regions in cis to ordered domains will be highlighted.

2154-Plat

The C-Terminal V5 Domain of Protein Kinase Cα is a Multi-Functional Intrinsically Disordered Protein Module

Yuan Yang, Tatyana I. Igumenova.
Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA.

Protein Kinase C (PKC) family of isozymes regulate a multitude of signaling pathways that control cell growth, differentiation, and apoptosis. The extreme C-terminal V5 domain has been identified as a key player in maturation, activation and down-regulation of PKCs, but the molecular basis of these events remains poorly understood. We report the first large-scale purification and NMR characterization of the V5 domain from PKCα (V5α) and its phosphorylation-mimicking variant. The combined analysis of NMR chemical shifts and circular dichroism data revealed that both V5α constructs are intrinsically disordered protein domains. Unexpectedly, we found that V5α has a propensity to partition into membrane mimetics acquiring a partial helical structure. Our data suggest that V5α anchors its parent enzyme to membranes during the maturation process.

Using NMR techniques, we obtained direct evidence that V5α interacts with another domain of PKCα – the C2 regulatory domain. This interaction is mediated by the phosphorylated hydrophobic motif of V5α and is enhanced by Ca^{2+}. Similarly, the affinity of C2 to Ca^{2+} is enhanced in the presence of the phosphorylated hydrophobic motif. These findings indicate that V5α may function as an intra-molecular protein interaction module that sensitizes PKCα to Ca^{2+} ions.

The third aspect of V5α function pertains to its interactions with Pin1, a peptidyl-prolyl isomerase that has been implicated in the down-regulation of conventional PKCs. Pin1 catalyzes proline isomerization of the phosphorylated Ser/Thr-Pro motif. Our preliminary NMR data demonstrate that Pin1 interacts with the hydrophobic motif of V5α, which is a non-canonical site that lacks a proline after the phosphorylated serine. Our data support the hypothesis that V5α serves as a phosphorylation-dependent docking site for Pin1.

Acknowledgments: This work was supported by the Welch Foundation (A-1784).

2155-Plat

C-Terminal ERK D- (and F-Like) Domains Link the Na^+/H^+ Exchanger NHE1 to ERK2 Phosphorylation and Regulation via Scaffolding

Ruth Hendus-Altenberger, Jeff Schnell, Elena Pedraz,
Jonas Marstrand Lacour, Jane Johansen, Stine F. Pedersen,
Birthe B. Kragelund.
Department of Biology, University of Copenhagen, Copenhagen N, Denmark.

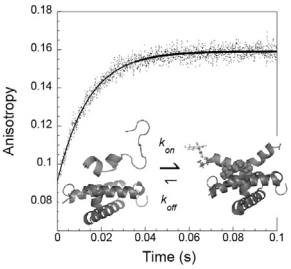
Increased activity of extracellular signal regulated kinase (ERK)1/2 plays a central role in cancer pathology. More recently, the membrane protein Na^+/H^+ exchanger 1 (NHE1) has also been assigned important roles in cancer development and chemotherapy resistance. Using a combination of fluorescence spectroscopy, heteronuclear NMR analysis, and protein engineering we characterized the interaction of the intrinsically disordered human (h) NHE1 C-terminal cytoplasmic tail (NHE1Cdt) and ERK2 in vitro, and found that NHE1Cdt and inactive ERK2 physically interact through exploitation of both D- and F-like domains of NHE1. Using active kinases we find that NHE1 is specifically phosphorylated by ERK2 and not MEK2 at several sites of importance for regulation of ERK2 activity and/or for NHE1 activity. We additionally found that NHE1 serves as a scaffold for MEK2-induced activation of ERK2. Mutating the D-domains in full-length hNHE1 and expressing wt and mutant NHE1s in epithelial cells lacking endogenous NHE1 evaluated the functional effects of NHE1-ERK interaction. Wt- and D-domain mutant hNHE1s appeared to target normally to the plasma membrane. D-domain mutation however abolished EGF-induced ERK activation, and D domains appeared to be required for full ERK activity. In contrast, net acid extrusion capacity after an acid load was similar in cells expressing D-domain mutant- and wt hNHE1s. In conclusion, ERK physically interacts with hNHE1 and this appears to regulate ERK activity via scaffolding.

2156-Plat

Speed Dating with KIX: A Single Domain that has Many Partners

Sarah L. Shammas, Jane Clarke.
Chemistry, University of Cambridge, Cambridge, United Kingdom.

IDPs are overrepresented in processes such as signalling and transcription, where proteins often interact with a range of partners. One much-studied key hub protein is the coactivator CBP/p300, whose folded KIX domain binds to a number of different intrinsically disordered transcription factors. The interaction of KIX with several of its ligands has been well studied by equilibrium methods, and structural information is available for many of the complexes. By careful control and consideration of experimental conditions such as temperature and ionic strength we have been able to perform kinetic studies that reveal the mechanism of the association reaction of KIX with cMyb; the fastest protein-protein interaction yet reported. Furthermore, through comparative studies with several binding partners we shed light on an important outstanding question in the IDP field: what is the advantage of disorder to a protein?



2157-Plat

Molecular Simulations of the Dynamics of Disordered Proteins

W. Wendell Smith[1], Po-Yi Ho[1], Elizabeth Rhoades[1,2], Corey O'Hern[1,3].
[1]Physics, Yale University, New Haven, CT, USA, [2]Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [3]Mechanical Engineering, Yale University, New Haven, CT, USA.

Intrinsically disordered proteins (IDPs) do not possess well-defined three-dimensional structures in solution under physiological conditions. We develop all-atom, united-atom, and coarse-grained Langevin dynamics simulations for the IDPs α-, β-, and γ-synuclein and microtubule-associated protein tau (MAPT) that include geometric, attractive hydrophobic, and screened electrostatic interactions and are calibrated to the inter-residue separations measured in recent smFRET experiments. We find that all four proteins are disordered with conformational statistics that are intermediate between random walk and collapsed globule behavior and demonstrate close resemblance to the known experimental data. We find that the hydrophobic interactions strongly influence the dynamics of these proteins, and electrostatics only play a role when charges fluctuate over more than several residues as for MAPT. We also investigate the propensity of α-synuclein and MAPT to aggregate and form oligomers, and compare to our previous results with α-synuclein monomers.

2158-Plat

The Protection of Membranes from Cold-Stress: A Structural Study of the Intrinsically Disordered Dehydrin Bound to Micelles and Liposomes

Steffen P. Graether, Matthew Clarke, Josephine Warnica, John Atkinson, Jeffrey Madge.
University of Guelph, Guelph, ON, Canada.

Plants produce numerous proteins to respond to cold and drought. One of these responses includes the expression of dehydrins, a family of proteins whose upregulation protects plants from various abiotic stresses. Using the 48-residues K2 dehydrin from Vitis riparia, we characterize the binding interaction and the structural changes that occur as this protein goes from a disordered protein in solution to one that contains weak alpha-helices after binding to liposomes and SDS micelles. We show that the addition of K2 protein to liposomes prevents their fusion after being frozen and thawed. We subsequently use SDS micelles as a membrane mimetic to probe what structural changes occur upon binding. We observe that a maximal change in helical content occurs well before the critical micelle concentration of SDS is reached. A series of ensemble structures were calculated for the free and micelle-bound forms of the protein using several NMR restraints. The conserved regions of the protein, containing several lysines, are shown to interact with the membrane surface, while a poorly conserved region of the protein maintains its high level of flexibility. Using chemical shift perturbation data and paramagnetic relaxation enhancement probes, we suggest which residues may be involved with membrane binding, and present a structural model of the protein bound to a membrane surface.